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Inhibition of Influenza Replication through the Restrictive Factors Modulation by CCR5 and CXCR4 Receptor Ligands

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Abstract : The exposure of A(H1N1)pdm09-infected epithelial cells (HeLa) to HIV-1 viral particles, or its gp120, enhanced interferon-induced transmembrane protein (IFITM3) content, a viral restriction factor (RF), resulting in a decrease in influenza replication. The gp120 binds to CCR5 (R5) or CXCR4 (X4) cell receptors during HIV-1 infection. Then, it is possible that the endogenous ligands of these receptors also modulate the expression of IFITM3 and other cellular factors that restrict influenza virus replication. Thus, the aim of this study is to analyze the role of cellular receptors R5 and X4 in modulating RFs in order to inhibit the replication of the influenza virus. A549 cells were treated with 2x effective dose (ED50) of endogenous R5 or X4 receptor agonists, CCL3 (20 ng/ml), CCL4 (10 ng/ml), CCL5 (10 ng/ml) and CXCL12 (100 ng/mL) or exogenous agonists, gp120 Bal-R5, gp120 IIIB-X4 and its mutants (5 µg/mL). The interferon α (10 ng/mL) and oseltamivir (60 nM) were used as a control. After 24 h post agonists exposure, the cells were infected with virus influenza A(H3N2) at 2 MOI (multiplicity of infection) for 1 h. Then, 24 h post infection, the supernatant was harvested and, the viral titre was evaluated by qRT-PCR. To evaluate IFITM3 and SAM and HD domain containing deoxynucleoside triphosphate triphosphohydrolase 1 (SAMHD1) protein levels, A549 were exposed to agonists for 24 h, and the monolayer was lysed with Laemmli buffer for western blot (WB) assay or fixed for indirect immunofluorescence (IFI) assay. In addition to this, we analyzed other RFs modulation in A549, after 24 h post agonists exposure by customized RT² Profiler Polymerase Chain Reaction Array. We also performed a functional assay in which SAMHD1-knocked-down, by single-stranded RNA (siRNA), A549 cells were infected with A(H3N2). In addition, the cells were treated with guanosine to assess the regulatory role of dNTPs by SAMHD1. We found that R5 and X4 agonists inhibited influenza replication in 54 ± 9%. We observed a four-fold increase in SAMHD1 transcripts by RFs mRNA quantification panel. After 24 h post agonists exposure, we did not observe an increase in IFITM3 protein levels through WB or IFI assays, but we observed an upregulation up to three-fold in the protein content of SAMHD1, in A549 exposed to agonists. Besides this, influenza replication enhanced in 20% in cell cultures that SAMDH1 was knockdown. Guanosine treatment in cells exposed to R5 ligands further inhibited influenza virus replication, suggesting that the inhibitory mechanism may involve the activation of the SAMHD1 deoxynucleotide triphosphohydrolase activity. Thus, our data show for the first time a direct relationship of SAMHD1 and inhibition of influenza replication, and provides perspectives for new studies on the signaling modulation, through cellular receptors, to induce proteins of great importance in the control of relevant infections for public health.

Keywords : chemokine receptors, gp120, influenza, virus restriction factors
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